FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **PA-9816**

U S APPLICATION NO. (IF KNOWN, SEE ~~37~~ CFR): **09/674,616**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/01550	**14 May 1999**	**15 May 1998**

TITLE OF INVENTION

Labelled Glutamine and Lysine Analogues

APPLICANT(S) FOR DO/EO/US

A. Storey, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☐ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☒ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☐ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

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01 FC:154 130.00 CH

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Additional Copy of this transmittal for charging purposes

Copy of the Notification of Missing Requirements

Certificate of Mailing

Return Postcard

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
To be assigned 09/674616	PCT/GB99/01550	PA-9816

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO **$970.00**	
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO **$840.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$690.00**	
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**	
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$96.00**	
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$840.00**
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	19 - 20 =	0	x $18.00	**$0.00**	
Independent claims	3 - 3 =	0	x $80.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
	TOTAL OF ABOVE CALCULATIONS =			**$840.00**	
	Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**. ☐			**$0.00**	
	SUBTOTAL =			**$840.00**	
	Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +			**$0.00**	
	TOTAL NATIONAL FEE =			**$840.00**	
	Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐			**$0.00**	
	TOTAL FEES ENCLOSED =			**$840.00**	
				Amount to be: refunded	$
				charged	$

☐ A check in the amount of to cover the above fees is enclosed.

☒ Please charge my Deposit Account No. **500-588** in the amount of **$840.00** to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **500-588** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

Royal N. Ronning, Jr.
NAME

32,529
REGISTRATION NUMBER

1 November 2000
DATE

PA-9816

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	A. Storey, et al.	Group Art Unit:	To be assigned
Serial number:	To be assigned	Examiner:	To be assigned
Filed:	1 November 2000		
For:	Labelled Glutamine and Lysine Analogues		

FIRST PRELIMINARY AMENDMENT

Honorable Assistant Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Please consider the following amendments and remarks in connection with the prosecution of the captioned application, which claims priority to International Application number PCT/GB99/01550 filed May 14, 1999.

IN THE CLAIMS

In Claim 5, line 1, please delete "Claims 1 to 4," and substitute -- Claim 1 -- therefor

In Claim 7, line 1, please delete "any one of Claims 1-6" and substitute--Claim 1--therefor.

In Claim 8, line 1, please delete "any one of Claims 1-7" and substitute--Claim 1--therefor.

In Claim 10, line 1, please delete "or Claim 9".

In Claim 13, lines 2, please delete "any one of Claims 1-12" and substitute--Claim 1--therefor.

Please amend Claim 14 as follows:

Claim 14 (once amended) A kit comprising the compound of [any one of Claims 1-9] Claim 1 [useful]for use in the preparation of [the] metal complexes [of any one of Claims 10-12].

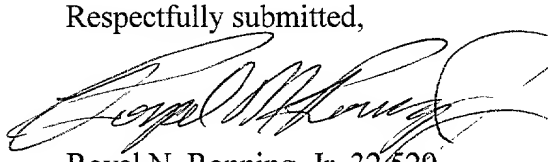
In Claim 19, lines 1-2, please delete "or Claim 18".

REMARKS

Claims 1-19 are pending in the captioned application, Claims 5, 7, 8, 10, 13, 14, and 19 to delete multiple dependencies. Applicants respectfully assert that the amendments are fairly based on the specification, and respectfully request their entry.

Applicants respectfully submit that their Claims 1-19, as amended, are in allowable form and earnestly solicit their allowance.

Respectfully submitted,



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LABELLED GLUTAMINE AND LYSINE ANALOGUES

The present invention relates to a class of compounds useful in the diagnosis of sites of venous and arterial thrombosis, embolism or infection, pharmaceutical formulations containing them, their use in the diagnosis of disease and methods for their preparation.

Prior approaches to thrombus imaging radiopharmaceuticals include radiolabelled fibrinogen or plasminogen; radiolabelled fragment E_1 of human fibrin; radiolabelled plasminogen activators such as tissue plasminogen activator (t-PA) and labelled anti-fibrin antibodies. Methods based on the detection of sites of platelet accumulation such as the administration of radiolabelled platelets (e.g. using ^{111}In oxine) or radiolabelled anti-platelet antibodies have also been described. More recent efforts have focused on radiolabelled peptides or polypeptides such as the cell adhesion motif RGD (where R, G and D are the standard abbreviations for the amino acids arginine, glycine and aspartic acid respectively); platelet factor 4 or fragments thereof or anticoagulant peptides such as disintegrins.

Factor XIII is a plasma glycoprotein which is present in blood and certain tissues in a catalytically inactive (or zymogen) form. Factor XIII is transformed into its active form Factor XIIIa by thrombin in the presence of calcium ions. Factor XIIIa is also known as plasma transglutaminase, fibrinoligase or fibrin-stabilising factor. The final step in the formation of a blood clot is the covalent crosslinking of the fibrin which is formed by the proteolytic cleavage of fibrinogen by thrombin. Fibrin molecules align and the enzyme Factor XIIIa catalyses covalent crosslinking of the NH_2 and $CONH_2$ groups of lysyl and glutaminyl residues respectively giving structural rigidity to the blood clot. The crosslinking stabilises the fibrin clot structure and confers resistance to fibrinolysis. The crosslink formation is

an important facet of normal blood coagulation and wound healing as well as pathological conditions such as thrombosis. As atherothrombotic brain infarctions are a common sub-type of stroke, Factor XIIIa substrates may allow diagnosis of stroke. It may also be implicated in atherosclerosis, inflammatory processes, tumour growth and metastasis. WO 91/16931 discloses that radiolabelled analogues of Factor XIII (in which the active site has been inactivated by amino acid substitution) are useful as thrombus imaging radiopharmaceuticals.

Factor XIIIa is also known to catalyse the incorporation of low molecular weight amines into the γ-glutamine sites of proteins. Similarly Factor XIIIa also catalyses the incorporation of low molecular weight glutamine analogues into lysyl residues. Thus such low molecular weight amines (or glutamine analogues) function as competitive inhibitors of the Factor XIIIa-induced lysyl/glutaminyl crosslinking of proteins. A range of synthetic amines have been described which are competitive inhibitors of the uptake of labelled putrescine (1,4-butanediamine) into N,N'-dimethylcasein catalysed by pig liver transglutaminase [L.Lorand *et al.*, Biochem., 18, 1756(1979)].

WO 89/00051 (Cytrx Biopool Ltd.) claims a method for targeting fibrin deposits using a labelled compound which is covalently bound to fibrin by Factor XIIIa. The fibrin binding compound is stated to be "any peptide that is a substrate for the blood enzyme commonly known as Factor XIIIa". Preferred peptides are said to include the tetrapeptide sequence -Asn-Gln-Glu-Gln- (or NQEQ in standard amino acid abbreviation notation). Also disclosed is the 12-mer peptide sequence from the NH_2 terminus of the alpha-2 antiplasmin enzyme:
NH_2-Asn-Gln-Glu-Gln-Val-Ser-Pro-Leu-Thr-Leu-Thr-Leu-Leu-Lys-OH
together with a synthetic analogue:
NH_2-Asn-Gln-Glu-Gln-Val-Ser-Pro-Tyr-Thr-Leu-Thr-Leu-Leu-Lys-OH,
(denoted NQEQVSPLTLTLLK and NQEQVSPYTLTLLK respectively). The

latter was radiolabelled with ^{125}I and shown to be taken up in thrombin clots *in vitro*.

It has now been discovered that synthetic analogues of lysine and glutamine labelled with a suitable detectable moiety can also function as substrates for the enzyme Factor XIIIa. The use of suitable protecting groups provides compounds which are less susceptible to *in vivo* metabolism especially by peptidases, and are hence more useful targeting agents.

The present invention provides the following compounds:

$$Y\text{-}(CR_2)_n\text{-}X\text{-}NHJ$$

where:

X is C=O or CR_2;

n is an integer of value 1 to 6;

Y is $L(A)_m$- or R^1R^2CR- where L is a metal complexing agent, A is $-CR_2-$, $-CR=CR-$, $-C\equiv C-$, $-NRCO-$, $-CONR-$, $-SO_2NR-$, $-NRSO_2-$, $-CR_2OCR_2-$, $-CR_2SCR_2-$, $-CR_2NRCR_2-$, a C_{4-8} cycloheteroalkylene group, a C_{4-8} cycloalkylene group, a C_{5-12} arylene group, a C_{3-12} heteroarylene group or a polyalkyleneglycol, polylactic acid or polyglycolic acid moiety;

m is an integer of value 0 to 10;

where one of R^1 and R^2 is $-NH(B)_pZ^1$ and the other is $-CO(B)_qZ^2$ where

p and q are integers of value 0 to 45, and

each B is independently chosen from Q or an amino acid, where Q is a cyclic peptide;

Z^1 and Z^2 are protecting groups;

J and each R group are independently chosen from H, C_{1-4} alkyl, C_{1-4} alkenyl, C_{1-4} alkynyl, C_{1-4} alkoxyalkyl or C_{1-4} hydroxyalkyl;

with the provisos that:

(i) the total number of amino acid residues in the R^1 and R^2 groups does not exceed 45;

(ii) when X is CR_2, then Y is $-CRR^1R^2$ and Z^2 is a metal complexing agent;

(iii) when Y is $-CRR^1R^2$ then at least one of R^1 and R^2 bears at least one detectable moiety.

The invention also includes kits for the preparation of the above compounds labelled with a detectable moiety, and the use of these and related compounds in the diagnosis or therapy of thrombosis, embolism, atherosclerosis, inflammation or cancer.

By the term "cyclic peptide" is meant a sequence of 5 to 15 amino acids in which the two terminal amino acids are bonded together by a covalent bond which may be a peptide or disulphide bond or a synthetic non-peptide bond such as a thioether, phosphodiester, disiloxane or urethane bond.

By the term "amino acid" is meant an *L*- or *D*-amino acid, amino acid analogue or amino acid mimetic which may be naturally occurring or of purely synthetic origin, and may be optically pure, i.e. a single enantiomer and hence chiral, or a mixture of enantiomers. Preferably the amino acids of the present invention are optically pure. By the term "amino acid mimetic" is meant synthetic analogues of naturally occurring amino acids which are isosteres, i.e. have been designed to mimic the steric and electronic structure of the natural compound. Such isosteres are well known to those skilled in the art and include but are not limited to depsipeptides, retro-inverso peptides, thioamides, cycloalkanes or 1,5-disubstituted tetrazoles [see M. Goodman, Biopolymers, 24, 137, (1985)].

By the term "protecting group" is meant a biocompatible group which inhibits or suppresses *in vivo* metabolism of the peptide or amino acid at the amino or carboxyl terminus. Such groups are well known to those skilled in the art and are suitably chosen from, for the amine terminus (Z^1): acetyl, Boc (where Boc is *tert*-butyloxycarbonyl), Fmoc

(where Fmoc is fluorenylmethoxycarbonyl), benzyloxycarbonyl, trifluoroacetyl, allyloxycarbonyl, Dde [i.e. 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl], Npys (i.e. 3-nitro-2-pyridine sulfenyl), or a metal complexing group; and for the carboxyl terminus (Z^2): a carboxamide, *tert*-butyl ester, benzyl ester, cyclohexyl ester, amino alcohol or a metal complexing group. Preferably the protecting group is a metal complexing group, most preferably it is a metal complexing group bound to a metal i.e. a metal complex. The carboxyl terminus of peptides is particularly susceptible to *in vivo* cleavage by carboxypeptidase enzymes. Consequently, the metal complexing group or metal complex is preferably attached at the carboxyl terminus. When either R^1 is $-NH(B)_{p-1}QZ^1$ or R^2 is $-CO(B)_{q-1}QZ^2$ then the protecting group may be the covalent bond which closes the cyclic peptide (Q) ring.

A "detectable moiety" is a moiety which emits a signal or is suitable for diagnostic imaging of the human body and may be a radioisotope for radiopharmaceutical imaging or therapy, a paramagnetic metal or species for MRI contrast imaging, a radiopaque group or metal for X-ray contrast imaging, a gas microbubble ultrasound contrast agent or a suitable dye for detection by external light imaging. Preferably, the imaging moiety is a metal ion, most preferably it is a radiometal.

When Y is $-CRR^1R^2$, then preferably either one or both of R^1 and R^2 comprises one or more peptide fragments of α_2-antiplasmin, fibronectin or beta-casein, fibrinogen or thrombospondin. Such peptide fragments comprise at least 3 and preferably 4 – 20 amino acid residues. When Y is $-CRR^1R^2$ and $-(CR_2)$-X-NHJ is $-(CH_2)_4NH_2$ (i.e. the amino acid side chain of lysine), then preferably either one or both of R^1 and R^2 comprises one or more such peptide fragments of α_2-antiplasmin, fibronectin or beta-casein. The amino acid sequences of α_2-antiplasmin, fibronectin, beta-casein, fibrinogen and thrombospondin can be found in the following references: α_2-antiplasmin precursor [M.Tone *et al.*,

J.Biochem, 102, 1033, (1987)]; beta-casein [L.Hansson *et al*, Gene, 139, 193, (1994)]; fibronectin [A.Gutman *et al*, FEBS Lett., 207, 145, (1996)]; thrombospondin-1 precursor [V.Dixit *et al*, Proc. Natl. Acad. Sci., USA, 83, 5449, (1986)]; R.F.Doolittle, Ann. Rev. Biochem., 53, 195, (1984).

Preferably the amino acid sequence is taken from the N-terminus of:

(i) α_2-antiplasmin,

i.e. NH_2-Asn-Gln-Glu-Gln-Val-Ser-Pro-Leu-Thr-Leu-Thr-Leu-Leu-Lys-OH or variants of this in which one or more amino acids have been exchanged, added or removed such as:

NH_2-Asn-Gln-Glu-Gln-Val-Ser-Pro-Leu-Thr-Leu-Thr-Leu-Leu-Lys-Gly-OH,

NH_2-Asn-Gln-Glu-Ala-Val-Ser-Pro-Leu-Thr-Leu-Thr-Leu-Leu-Lys-Gly-OH,

NH_2-Asn-Gln-Glu-Gln-Val-Gly-OH; or

(ii) casein

ie. Ac-Leu-Gly-Pro-Gly-Gln-Ser-Lys-Val-Ile-Gly.

When the compound of the present invention is a peptide, i.e. Y is R^1R^2CR- the number of amino acid residues is preferably 2 to 30, most preferably 3 to 20, especially 3 to 15.

Preferred compounds have J equal H, i.e. terminate in an NH_2 group. X is preferably C=O, i.e. compounds of formula Y-$(CR_2)_n$-$CONH_2$ are preferred. Most preferred compounds are of formula Y-$(CR_2)_x$-$(CH_2)_2CONH_2$ or Y-$(CR_2)_y$-$(CH_2)_4NH_2$ where x is an integer of value 0 to 4, and y is an integer of value 0 to 3. Compounds having the same side chain as glutamine, i.e. glutamine analogues of formula Y-$(CR_2)_x$-$(CH_2)_2CONH_2$ are especially preferred.

Suitable non-metallic radioisotopes for use in the present invention include but are not limited to: radioiodine such as ^{123}I, ^{125}I, ^{131}I, preferably ^{123}I; positron emitters such as ^{18}F, ^{11}C or ^{75}Br and isotopes for therapy e.g. ^{211}At.

The compounds of this invention which comprise metal

complexing agents preferably have only a single type of targeting molecule attached, i.e. the $-(CR_2)_n$-X-NHJ substituent. Other substituents on the complexing agent may be present, but the $-(CR_2)_n$-X-NHJ substituent is the one which is expected to be primarily responsible for the biolocalisation properties. Metal complexes of the present invention may contain one or more metal ions which may be the same or different. Thus in some circumstances polynuclear complexes may have advantageous properties such as certain metal clusters which have superparamagnetic properties and are hence particularly useful as MRI contrast agents. Preferred metal complexes of the present invention involve only a single metal ion. When the metal of the metal complex is a radiometal, it can be either a positron emitter (such as ^{68}Ga or ^{64}Cu) or a γ-emitter such as ^{99m}Tc, ^{111}In, ^{113m}In or ^{67}Ga. Suitable metal ions for use in MRI are paramagnetic metal ions such as gadolinium(III) or manganese(II). Most preferred radiometals for diagnostic imaging are γ-emitters, especially ^{99m}Tc. Metal complexes of certain radionuclides may be useful as radiopharmaceuticals for the radiotherapy of various diseases such as cancer or the treatment of thrombosis or restenosis. Useful radioisotopes for such radiotherapeutic applications include: ^{90}Y, ^{89}Sr, ^{67}Cu, ^{186}Re, ^{188}Re, ^{169}Er, ^{153}Sm and ^{198}Au. Whichever metal complex is chosen, it is strongly preferred that it is bound to the Factor XIIIa substrate in such a way that it does not undergo facile metabolism in blood with the result that the metal complex is cleaved from the Factor XIIIa substrate before the labelled Factor XIIIa substrate reaches the desired *in vivo* site to be imaged. The Factor XIIIa substrate is therefore preferably covalently bound to the metal complexes of the present invention.

These metal ions are complexed using a metal complexing agent, or more preferably a chelating agent. The chelating agents comprise 2-10 metal donor atoms covalently linked together by a non-coordinating backbone. Preferred chelating agents have 4-8 metal donor

atoms and have the metal donor atoms in either an open chain or macrocyclic arrangement or combinations thereof. Most preferred chelating agents have 4-6 metal donor atoms and form 5- or 6-membered chelate rings when coordinated to the metal centre. Such polydentate and/or macrocyclic chelating agents form stable metal complexes which can survive challenge by endogenous competing ligands for the metal *in vivo* such as transferrin or plasma proteins. Alternatively, it is possible to use monodentate complexing agents that form stable complexes with the desired metal ion even though they do not form chelate rings upon metal coordination. Examples of known complexing agents of this kind, which are particularly suitable for use with ^{99m}Tc, are hydrazines, phosphines, arsines, or isonitriles.

Examples of suitable chelating agents are bidentate such as diamines or diphosphines, tridentate such as monoaminedithiols, or tetradentate such as diaminedioximes (US 4615876) or such ligands incorporating amide donors (WO 94/08949); the tetradentate ligands of WO 94/22816 ; N_2S_2 diaminedithiols, diamidedithiols or amideaminedithiols; N_3S thioltriamides; N_4 ligands such as tetraamines, macrocyclic amine or amide ligands such as cyclam, oxocyclam (which forms a neutral technetium complex) or dioxocyclam; or dithiosemicarbazones. The above described ligands are particularly suitable for technetium, but are useful for other metals also. Other suitable ligands are described in Sandoz WO 91/01144, which includes ligands which are particularly suitable for indium, yttrium and gadolinium, especially macrocyclic aminocarboxylate and aminophosphonic acid ligands. Ligands which form non-ionic (i.e. neutral) metal complexes of gadolinium are known and are described in US 4885363. The ligand may also comprise a short sequence of amino acids such as the Cys/amino acid/Cys tripeptide of WO 92/13572 or the peptide ligands described in EP 0719790 A2.

Preferred chelating agents have the formula

$$RN[(CR_2)_aN(CR_2)_bCR=NOH]_2$$

where each a is 2 or 3

each b is 1 or 2

one R is aminoalkylene through which the chelating agent is joined to the rest of the molecule

each other R is independently H, $C_1 - C_{10}$ hydrocarbyl, alkoxy, alkoxyalkyl, amine, amide, hydroxyl, hydroxyalkyl or carboxylate or two R groups taken together with the atoms to which they are attached form a carboxylic, heterocyclic saturated or unsaturated ring.

Non-peptide-based metal chelating agents provide improved control over attachment and release of metal ions, and are preferred.

The invention also includes a peptide fragment of α_2-antiplasmin, fibronectin, beta-casein, tetanus, amyloid, trappin or polyglutamine, said peptide fragment containing 3 – 45 amino acid residues and carrying a terminal metal complexing agent.

It is well known to prepare chelating agents which have attached thereto a functional group ("bifunctional chelates"). Functional groups which have been attached to chelating agents include: amine, carboxylic acid, cyanate, thiocyanate, maleimide and active ester such as N-hydroxysuccinimide. Examples of chelate-amine conjugates for diaminedioxime ligands are given in WO 95/19187. When the desired Factor XIIIa substrate functionality is an amine, the ligands of the present invention can be prepared by reaction of a bifunctional compound which contains both an amine group (preferably protected by use of suitable protecting groups known to those skilled in the art), and a reactive group such as a sulphonyl chloride, acid chloride, active ester or an alkyl/benzyl halide. The reactive group can then be coupled to either the pendant

amine group of a bifunctional chelate, or used to derivatise one or more of the amine donor atoms of a N-containing ligand. Alternatively, a mono-protected diamine could be reacted with a bifunctional chelate with a pendant active ester or carboxyl group to give the protected amine group linked to the ligand system *via* an amide bond. In both synthetic routes outlined above, the resulting ligand-protected amine conjugate is then deprotected under suitable conditions to give the desired amine-functionalised ligand. When the desired Factor XIIIa substrate functionality is a carboxamide group, the desired ligands can be prepared e.g. by reaction of a omega-haloalkyl carboxamide of suitable chain length with a bifunctional chelate with a pendant amine group, giving the desired carboxamide-linked ligand.

The metal complexes of the present invention may be prepared by reacting a solution of the metal in the appropriate oxidation state with the ligand at the appropriate pH. The solution may preferably contain a ligand which complexes weakly to the metal (such as chloride, gluconate or citrate) i.e. the metal complex is prepared by ligand exchange or transchelation. Such conditions are useful to suppress undesirable side reactions such as hydrolysis of the metal ion. When the metal ion is ^{99m}Tc, the usual starting material is sodium pertechnetate from a ^{99}Mo generator. Technetium is present in ^{99m}Tc-pertechnetate in the Tc(VII) oxidation state, which is relatively unreactive. The preparation of technetium complexes of lower oxidation state Tc(I) to Tc(V) therefore usually requires the addition of a suitable reducing agent such as stannous ion to facilitate complexation. Further suitable reductants are described below.

The metal complex should also preferably exhibit low non-specific blood background.

Thus the present invention relates mainly to diagnostic agents for imaging sites in the mammalian body where the enzyme Factor XIII is activated and blood proteins such as fibrin or collagen are deposited.

The present agents are particularly useful for the diagnostic imaging of the human body. The agents comprise substrates for the enzyme Factor XIIIa which are labelled with a metal complex suitable for external imaging such as a radiometal (for scintigraphy) or a paramagnetic metal ion (for MRI). The metal complex of the present invention has a pendant amino or carboxamide functional group which is available for covalent linking to protein glutamyl carboxamide or lysyl amine groups respectively by the enzyme Factor XIIIa. The intimate relationship of fibrin and Factor XIIIa highlights the potential use of the agents of the present invention for the diagnosis of disease states where there is both fibrin deposition or accumulation and activation of Factor XIII. Increased fibrin deposition is known to be characteristic of diseases such as thrombosis, atherosclerosis, fibrotic liver, and disseminated intravascular coagulation. Fibrin is also deposited at sites of tissue inflammation associated with many disease processes, such as infection, autoimmune disease or cancer. Factor XIII and tissue transglutaminase are activated during known physiological conditions. During apoptosis and generation of new matrix protein structures elevated levels of the enzymes are seen. The present agents may thus also be used for the detection of apoptosis and diseases states such as arthritis where increased matrix protein deposition occurs. Since Factor XIII is activated at the site of interest *in vivo* (i.e. thrombus, embolism etc.) this provides a localisation mechanism for the metal complexes of the present invention. The covalently linked metal complexes can then be imaged externally by radionuclide scintigraphy or magnetic resonance imaging (MRI) hence providing a non-invasive means of diagnosing the disease site.

So far as the therapeutic aspects of this invention are concerned, the inventors have preliminary *in vivo* data (not reported in detail herein) indicating that clots produced in the presence of labelled peptides of the present invention (as per Example 17 below) are smaller

than those produced in the absence of the labelled peptide. On this basis it is proposed that the peptides herein defined, typically containing 4 – 30 e.g. about 10 amino acid residues, are effective as drugs for increasing the rate of clot lysis e.g. by acting as potent inhibitors of fibrin cross linking in clots. Thus the compounds disclosed have possible pharmaceutical uses as thrombolytic or anticoagulant drugs for therapy.

The present invention also relates to kits for the preparation of metal complexes linked to Factor XIIIa substrates. The kits are designed to give sterile products suitable for human administration, e.g. *via* injection into the bloodstream. Possible embodiments are discussed below. When the detectable moiety is ^{99m}Tc, the kit would comprise a vial containing the free ligand or chelating agent for the metal together with a pharmaceutically acceptable reducing agent such as sodium dithionite, sodium bisulphite, ascorbic acid, formamidine sulphinic acid, stannous ion, Fe(II) or Cu(I), preferably a stannous salt such as stannous chloride or stannous tartrate. Alternatively, the kit could contain a metal complex which, upon addition of the radiometal or paramagnetic metal, undergoes transmetallation (i.e. ligand exchange) giving the desired product. For ^{99m}Tc, the kit is preferably lyophilised and is designed to be reconstituted with sterile ^{99m}Tc-pertechnetate (TcO_4^-) from a ^{99m}Tc radioisotope generator to give a solution suitable for human administration without further manipulation.

The agents of the present invention may also be provided in a unit dose form ready for human injection and could for example be supplied in a pre-filled sterile syringe. When the detectable moiety is a radioactive isotope such as ^{99m}Tc, the syringe containing the unit dose would also be supplied within a syringe shield (to protect the operator from potential radioactive dose).

The above kits or pre-filled syringes may optionally contain further ingredients such as buffers; pharmaceutically acceptable

solubilisers (e.g. cyclodextrins or surfactants such as Pluronic, Tween or phospholipids); pharmaceutically acceptable stabilisers or antioxidants (such as ascorbic acid, gentisic acid or *para*-aminobenzoic acid) or bulking agents for lyophilisation (such as sodium chloride or mannitol).

The following examples illustrate the preparation of compounds of the present invention and their use in imaging. The syntheses of particular compounds of the present invention are given in Examples 1-9, their radiolabelling with ^{123}I or ^{99m}Tc in Examples 10-12. Compound 1 (prior art) is included as a comparative example. Evidence for increased plasma stability *in vitro* is given in Example 13. Evidence for uptake in blood clots *in vitro* and *in vivo* is given in Examples 15 and 17 respectively, with normal rat biodistribution of the radiolabelled compounds reported in Example 16.

The *in vitro* plasma stability of ^{123}I-Compound 1 is poor (see Example 13), presumably due to protease activity. The introduction of protecting groups at both the carboxy and amino termini as for radiolabelled Compounds 2-5 and 7-49 confers a substantial increase in plasma stability.

The majority of compounds tested exhibit high *in vitro* clot uptake and hence avidity for the clot. The other compounds are of lower potency with compounds 14, 16, 18, 31, 34, 36, 46 and 48 showing significant reduction in uptake. Removal of the Gln residue from position 2 of the α_2-antiplasmin derived sequences, as in compound 14, causes a large drop in uptake of this tracer, thus strongly suggesting that Gln-2 is an essential amino acid in this sequence type.

Details of the biodistribution in normal rats and in the fresh and aged clot models are given in Examples 16 and 17. The blood clearance rate of these compounds is relatively fast with biological half lives between 1-2 hours. The biodistribution of ^{99m}Tc-Compound 3 is given as a representative example, in this case the $t_{\frac{1}{2}}$ of 2h is estimated. The

rapid clearance from background tissues such as blood, lung, heart and muscle shows that the agent of the present invention possess favourable pharmacokinetics for imaging and shows their potential as radiodiagnostics. Although some hepatobiliary excretion is seen for these compounds, the main route of excretion is *via* the urinary tract.

Uptake into fresh and aged clots in the rat models for many of the radiolabelled compounds is very good (relative concentration or RC= 5-15), with clot to background tissue ratios very favourable for imaging (>5). Example 18 shows that ^{99m}Tc-Compound 5 is suitable for imaging clots in the rat model.

^{99m}Tc-Compounds 2 - 49 have improved plasma stability compared with ^{123}I-Compound 1 (RC = 1.5), which may be responsible for the improved *in vivo* clot uptake seen with these compounds.

Comparison of the clot uptake results of Example 17 for fresh and aged clots, shows that the present agents exhibit uptake which is constant and independent of the age of the clot. Thus such agents will have improved imaging capability for pre-existing clots, such as those found with pulmonary embolism.

EXPERIMENTAL

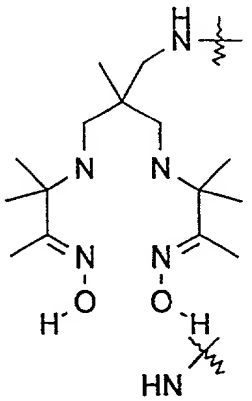
In the following table:

Z is benzyloxycarbonyl,

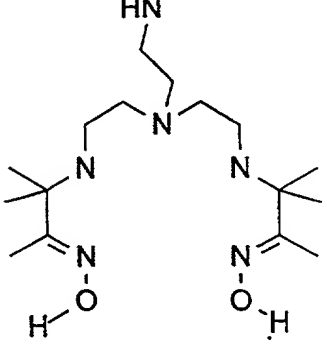
Fmoc is fluorenylmethoxycarbonyl,

Ac is acetyl,

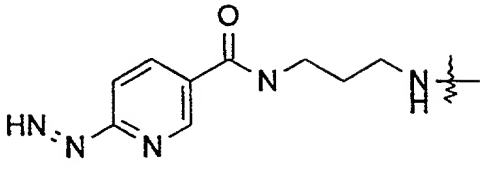
Pn44 is



Pn216 is



Hynic is



Peptide	Compound	MS Theor.	MS Expt.
NQEQVSPYTLLK	1	1419.6	1419.7[a]
Ac-NQEQVSPYTLLKG-NH_2	2	1517.7	1517.7
Ac-NQEQVSPYTLLKG-Pn44	3	1816.1	1816.2
Ac-NQEQVSPYTLLKG-Hynic	4	1709.7	1710.0
Ac-NQEQVSPYTLLKG-Pn216	5	1845.2	1845.2
NQEQVSPYTLLKG-Pn216	6	1803.1	1803.3
Z-NQEQVSPYTLLKG-Pn44	7	1908.2	1908.1
Z-NQEQVSPYTLLKG-Pn216	8	1937.2	1937.2
Fmoc-NQEQVSPYTLLKG-Pn216	9	2025.4	2025.4
$(CF_3)_2(C_6H_3)$- NQEQVSPYTLLKG-Pn216	10	2044.2	2045.2
Adamantoyl-NQEQVSPYTLLKG-Pn216	11	1966.0	1966.5
Ac-NQEAVSPYTLLKG-Pn44	12	1759.1	1759.0
Ac-NQEAVSPYTLLKG-Pn216	13	1788.1	1788.0
Ac-NAEAVSPYTLLKG-Pn216	14	1766.1	1765.8
Ac-NQQQVSPYTLLKG-Pn216	15	1844.2	1844.1
Ac-NQG-Pn44	16	656.8	656.6
Ac-NQEQVG-Pn44	17	1908.2	1908.1
Z-NQEQVSPYG-Pn216	18	1481.7	1481.7
Ac-NQEQVSPLTLLKG-Pn216	19	1795.2	1795.1
Ac-NQEQVSP-Nal(2)-TLLKG-Pn216	20	1879.3	1879.3
Ac-NQEQVSP(*p*Br-F)TLLKG-Pn216	21	1908.1	1908.9
Ac-NQEQVSP(I-Y)TLLKG-Pn216	22	1971.2	1972.4
Ac-NQEQVSP(I_2-Y)TLLKG-Pn216	23	2098.2	2099.5
Z-NQEQVSP(I-Y)TLLKG-Pn216	24	2063.2	2064.4
Z-NQEQVSP(I_2-Y)TLLKG-Pn216	25	2189.2	2190.5
Fmoc-NQEQVSP(I-Y)TLLKG-Pn216	26	2152.4	2153.4
Fmoc-NQEQVSP(I_2-Y)TLLKG-Pn216	27	2278.4	2279.3
Ac-NQEQVSPYTLL(*D*-K)G-Pn216	28	1845.2	1845.2
Ac-NQEQVSP(*D*-Y)TLL(*D*-K)G-Pn216	29	1845.2	1845.1
Ac-NQEQV(*D*-S)P(*D*-Y)TLL(*D*-K)G-Pn216	30	1845.2	1845.2

Peptide	Compound	MS Theor.	MS Expt.
Ac-NQEQ(*D*-V)(*D*-S)P(*D*-Y)TLL(*D*-K)G-Pn216	31	1845.2	1845.1
Ac-(*D*-N)QEQVSP(*D*-Y)TLL(*D*-K)G-Pn216	32	1845.2	1845.0
Ac-NQEQVSP(*D*-Y)TLL(*D*-K)βAla-Pn216	33	1859.2	1859.0
Ac-N-CH_2NH_2-QEQVSP(*D*-Y)TLL(*D*-K)G-Pn216	34	1831.2	1831.1
Ac-NQEQ(*D*-V)(*D*-S)(*D*-P)(*D*-Y)(*D*-T)(*D*-L)(*D*-L)(*D*-K)G-Pn44	35	1816.1	1815.8
Pn216-$CO(CH_2)_3CO$-G(*D*-K)(*D*-L)(*D*-L)(*D*-T)(*D*-Y)(*D*-P)(*D*-S)(*D*-V)NH_2	36	1916.2	1916.0
Fmoc-NQQQ(*D*-V)S(OMe)PLG-Pn216	37	1532.8	1532.7
Pn44-$CO(CH_2)_3CO$-NQEQVSPYTLLKG-NH_2	38	1887.7	1887.3
Pn216-$CO(CH_2)_3CO$-NQEQVSPYTLLKG-NH_2	39	1916.2	1916.3
Ac-NQEQVSPYTLLKG-$(PEG)_{3\ 4k}$-Pn44	40	5200-5600	5401[b]
Ac-NQEQVSPYTLLKG-$(PEG)_{10k}$-Pn216	41	12400-	12630[b]
Z-NQEQVSPYAAAAG-Pn216	42	1766.0	1765.9
Z-NQEQVSPYG$(CH_2)_{11}$(CO)-Pn216	43	1677.7	1679.8
Cyclo-[NQEQVSPYTLLKG]	44	1458.6	1458.3
Ac-LGPGQSKVIG-Pn44	45	1294.6	1294.4
p-EAQIVG-Pn44	46	1023.2	1023.0
Ac-LEFDTQSKNILG-Pn216	47	1733.0	1732.9
Ac-GQDPVKG-Pn216	48	1068.3	1068.0
Ac-YEVHHQKLVFFG-Pn216	49	1872.2	1872.3

All compounds except those denoted were analysed using ES+ mass spectrometry. Those compounds denoted by [a] were analysed by FAB and [b] by MALDI-TOF mass spectrometry.

Example 1: Syntheses of Compounds 1 and 2

The protected peptide Ac-Asn(Trt)-Gln(Trt)-Glu(OtBu)-Gln(Trt)-Val-Ser(tBu)-Pro-Tyr(tBu)-Thr(tBu)-Leu-Leu-Lys(Boc)-Gly-OH was assembled on a 2-chlorotrityl resin by anchoring Fmoc-Lys(Boc) to the resin, and then successive deprotections/coupling cycles with the appropriate protected amino acids (as described in P. Lloyd-Williams, F. Albericio and E. Girald; *Chemical Approaches to the Synthesis of Peptides and Proteins*, CRC Press, 1997). The title compound was obtained by cleavage using 0.1% TFA in dichloromethane, deprotection and purification by RP-HPLC (System A).

Example 2: Syntheses of Compounds 3-9, 12-21, 28-33, 35, 37, 42 and 45-49

The appropriate protected peptide was assembled as in Example 1 with the appropriate protected amino acids. The protected fragment was cleaved from the resin and then coupled with 6-aminomethyl-3,3,6,9,9-pentamethyl-4,8-diazaundecane-2,10-dione dioxime (prepared as described in WO 95/19187), 3,3,11,11-tetramethyl-7-aminoethyl-4,7,10-triazatridecane-2,12-dionedioxime (prepared as described in WO 98/31399) or 6-Boc-hydrazinopyridine-3-carboxylic acid N-hydroxysuccinimide ester (prepared as described in US patent 5,206,370) in solution using BOP as a coupling agent. The title compounds were obtained by deprotection in TFA/water/triethylsilane (90/5/5) and purified by RP-HPLC (System A).

Example 3: Syntheses of Compounds 22-27

In an Eppendorf vial compound 5, 8 or 9 (1mg), ammonium acetate buffer (400µl, 0.2M, pH4), sodium iodide (0.5eq, 15mg/10ml in 0.1M NaOH) and peracetic acid (1.5 eq, 0.1M solution) were added. The

reaction mixture was thoroughly mixed for 1 minute and the mono- and di-iodo products were separated and collected by preparative HPLC. The procedure was repeated to give sufficient quantities of the isolated products.

Example 4: Syntheses of Compounds 10 and 11

Fmoc-Asn(Trt)-Gln(Trt)-Glu(tBu)-Gln(Trt)-Val-Ser(tBu)-Pro-Tyr(tBu)-Thr(tBu)-Leu-Leu-Lys(Boc)-Gly (200 mg, 0.73 mmol), Pn216 (30 mg, 0.87 mmol) and HBTU (33 mg, 0.87 mmol) were dissolved in anhydrous DMF (2.5 ml). To the solution was added diisopropylethylamine (20 ml, 1.15 mmol) and the reaction mixture was stirred at room for 1.75 hours. The reaction mixture was then treated with piperidine (0.5 ml) and the mixture stirred at room temperature for 2 hours. The product was purified by semi-preparative HPLC to give a white solid (171 mg, 82%); ES^+-MS: m/z 952.40 ($M+3H^+$).

1-Adamantanecarboxylic acid or 3,5-bis(trifluoromethyl)benzoic acid (1.5 molar equivalent), the protected peptide Asn(Trt)-Gln(Trt)-Glu(tBu)-Gln(Trt)-Val-Ser(tBu)-Pro-Tyr(tBu)-Thr(tBu)-Leu-Leu-Lys(Boc)-Gly-Pn216 (1 molar equivalent) and HBTU (1.2-1.5 molar equivalent) were dissolved in anhydrous DMF (1 ml). Diisopropylethylamine (11 molar equivalents) was added and the reaction mixture was stirred at room temperature until the reaction was judged complete by HPLC. The protected peptide fragment was then treated with 95% trifluoroacetic acid in CH_2Cl_2. The reaction mixture was stirred at room temperature for 2 to 4 hrs. The product was purified from the reaction mixture by reversed phase HPLC.

Example 5: Synthesis of Compound 34

The protected peptide was synthesised as for example with the exception of the Fmoc-Asn(Trt)-$\Psi(CH_2NH)$-Gln(Trt)-OH which was

obtained according to the classical methodology for the synthesis of reduced peptide bonds, by reductive amination of Gln(Trt) with Fmoc-Asn(Trt) derived aldehyde (see G. Guichard *et al.*, *Peptide Res.*, **6**(3), 121, (1993) and references therein.

The resulting compound was prepared and purified as in example 2.

Example 6: Syntheses of Compounds 36, 38 and 39

The protected peptide was synthesised as for example 1 but on a Rink resin. After removal of the glycine N-protection, the peptide was reacted with glutaric anhydride whilst still on the resin. Activation with BOP/HOBt of the glutarate carboxylic acid and coupling with 6-aminomethyl-3,3,6,9,9-pentamethyl-4,8-diazaundecane-2,10-dione dioxime or 3,3,11,11-tetramethyl-7-aminoethyl-4,7,10-triazatridecane-2,12-dionedioxime on the resin gave the protected product. Cleavage with TFA/water (95/5) afforded the crude material which was purified using RP-HPLC (System A).

Example 7: Syntheses of Compounds 40 and 41

A solution of the required molecular weight α-N-(*tert*-butoxycarbonyl)-poly(ethylene glycol) amino-ω-succinimidyl carbonate and one molar equivalent of 6-aminomethyl-3,3,6,9,9-pentamethyl-4,8-diazaundecane-2,10-dione dioxime or 3,3,11,11-tetramethyl-7-aminoethyl-4,7,10-triazatridecane-2,12-dionedioxime in anhydrous tetrahydrofuran (5 ml) was refluxed for 5 hours under nitrogen. The reaction mixture was reduced *in vacuo* leading to a white solid which was purified by flash chromatography using isopropanol/ ammonia/ water 10:1:1 to give the title compound as a white solid. The Boc protecting group was removed using 37% HCl added dropwise to an ice-cold solution in methanol. The solution was then stirred at room temperature for 4 hours. The reaction mixture

was basified to pH ~10 by addition of 4M NaOH (4.18 ml). The product was isolated by semi-preparative HPLC (system D).

The solid from above was dissolved in DMF and to it was added the protected peptide fragment. Diisopropylethylamine and HBTU were added and the reaction mixture was stirred at room temperature till completion of the reaction. The product was purified by HPLC (system E) to give a colourless gum.

This gum was dissolved in dichloromethane (2 ml) and the solution treated with TFA (0.2 ml) for 5 hours. The reaction was basified with 1M NaOH (2 ml) and volatiles removed *in vacuo*. MeOH (2.5 ml) was added to the residue and the mixture filtered using an Acrodisc filter (LC13 PVDF 0.45 m). The product was isolated from the methanolic solution by HPLC (system E).

Example 8: Synthesis of Compound 43

12-*N*-Fmoc-aminododecanoic acid was coupled to ,3,11,11-tetramethyl-7-aminoethyl-4,7,10-triazatridecane-2,12-dionedioxime as described earlier. The Fmoc protecting group was removed by 20% piperidine in DMF and the product purified by HPLC (system F).

The above product was coupled with the protected peptide fragment and subsequently deprotected as described above. The product was purified by HPLC (system G).

Example 9: Synthesis of Compound 44

The partially deprotected peptide H-Asn(Trt)-Gln(Trt)-Glu(OtBu)-Gln(Trt)-Val-Ser(tBu)-Pro-Tyr(tBu)-Leu-Leu-Lys(Boc)-Gly-OH was assembled on a 2-chlorotrtyl chloride resin, by a Fmoc based strategy, by stepwise elongation with BOP/HOBt. The N-terminal protection was removed by piperidine treatment and partially protected peptide cleaved from the solid support by a 0.5% TFA in dichloromethane solution.

Cyclisation was carried out in solution at a 10mM concentration in DMF, with BOP as a condensation reagent, in the presence of solid sodium bicarbonate according to a known methodology (see for instance M. Rodriguez *et al.*, *Int. J. Pept. Protein Res.*, **35**, 441, 1990).

Final deprotection in a mixture of TFA/water/ethane dithiol (90/5/5) afforded the crude title compound which was purified by RP-HPLC (System A).

Example 10: I-123 labelling of Compounds 1-2 and Compound 44

Ammonium acetate buffer (200µl, 0.2M, pH 4.0) was added to the ligand solution (20µl, 20µg), and $Na^{127}I$ (10µl, 1.5µg) in an Eppendorf tube. The solution was mixed thoroughly and $Na^{123}I$ (5-50µl, 111MBq) was then added. The solution was mixed thoroughly prior to addition of PAA solution (10µl, 0.01M), further mixing followed. The activity of the preparation was measured. In all cases the required product was separated from reaction by-products and unlabelled substrates by HPLC.

Example 11: Tc-99m labelling of Compounds 3, 5-43, 45-49

A 0.1ml aliquot of the compound dissolved in H_2O (1mg/ml) was transferred to a nitrogen-filled 10ml glass vial together with deoxygenated saline (0.9% w/v, 1ml) and 0.035ml aqueous NaOH (0.1M). To this solution was added technetium generator eluate (1ml, approx. 0.4GBq) and then aqueous stannous chloride solution (0.1ml, ca.10µg). The labelling pH was 9.0-10.0. Vials were incubated at ambient laboratory temperature (15-25°C) for 30 minutes to effect labelling. The resulting preparation was either diluted to the desired radioactive concentration or HPLC purification was performed (System B) to remove unlabelled starting material and radioactive impurities prior to testing. After purification the

organic solvent was removed under vacuum and the sample was redissolved in about 5ml 0.1M phosphate buffer pH 7.4 to give a working concentration of 6-9MBq/ml. Radiochemical purity was assessed before use by the thin layer chromatography (TLC) system described below:

i) ITLC SG 2cm x 20cm eluted with 0.9% w/v saline

ii) Whatman No.1 2cm x 20cm eluted with 50 : 50 v/v acetonitrile : H_2O

The labelled substrates remain at, or close to, the origin in TLC system (i) and move close to the solvent front in system (ii). When analysed by appropriate detection equipment the radiochemical purity is typically in excess of 85% labelled compound.

Example 12: Tc-99m labelling of Compound 4

A 0.1ml aliquot of the compound dissolved in water (1mg/ml) was transferred to nitrogen-filled 10ml glass vial together with tricine dissolved in water (0.5ml, 37.5mg) and phosphinedynetris(benzene sulphonic acid)tris sodium salt dissolved in water (0.1ml, 10mg). To this solution was added technetium generator eluate (1ml, approx 0.4GBq) and then a solution of stannous chloride in 0.1M HCl (0.02ml, ca 2μg). The labelling pH was 4.5-5.5. Vials were incubated at 60°C for 30 minutes to effect labelling. Purification and assessment of radiochemical purity was carried out as in Example 10.

Example 13: *In vitro* plasma stability

To a portion of compound (50μl, 10MBq/ml) was added an equal volume of plasma (rat or human) or saline. The mixtures were incubated at 37°C and the stability measured by HPLC (system C) at 0, 30 and 120 minutes. The saline dilution acted as a control.

Compound	Species	% intact at 120 mins
^{123}I-Cmpd 1 (prior art)	Human	0
^{123}I-Cmpd 2	Human	98
	Rat	99
^{99m}Tc-Cmpds 3, 4, 5, 7, 12, 16,17; ^{123}I- Cmpd 44	Human	>90
	Rat	>90
^{99m}Tc-Cmpds 8-11, 13-15,19-21,28-36, 38, 39, 45-49	Rat	>90
^{99m}Tc-Cmpds 22-27	Rat	>60

Example 14: HPLC Systems

Flow Rate: 1ml/min in all systems.

System A

Column Waters C18 250x4.5mm. Particle size 4 microns

Gradient: Elution Profile 10-60%B in 25 min.

Eluent A: 0.1% aqueous TFA

Eluent B: 0.1% TFA in acetonitrile

System B

Column Waters C18 150x3.9mm. Particle size 4 microns

Gradient: Elution Profile 0-100%B in 22 min.

Eluent A: 0.1% aqueous TFA

Eluent B: 0.1% TFA in acetonitrile

System C

Column	Waters C18 150x3.9mm. Particle size 4 microns
Gradient:	Elution Profile 0-100%B in 20 min.
Eluent A:	50mM NH_4OAc buffer (pH 5.6)
Eluent B:	acetonitrile

System D

Column	Hamilton PRP-1, 305 mm x 7.0 mm;
Gradient:	Elution Profile 0-65%B in 10 min.
Eluent A:	5% aqueous ammonia
Eluent B:	acetonitrile

System E

Column	Hamilton PRP-1, 150 mm x 4.1 mm;
Gradient:	Elution Profile 0-100%B in 15 min.
Eluent A:	5% aqueous ammonia
Eluent B:	acetonitrile

System F

Column	Polymer Laboratories PLRP-S, 150 mm x 2.5 mm;
Gradient:	Elution Profile 0-100%B in 15 min.
Eluent A:	5% aqueous ammonia
Eluent B:	acetonitrile

System G

Column	Hamilton PRP-1, 150 mm x 4.1 mm;
Gradient:	Elution Profile 0-100%B in 15 min.
Eluent A:	0.1% aqueous TFA
Eluent B:	0.1% TFA in acetonitrile

Example 15: Incorporation into human plasma clots

Incorporation of radiolabelled substrates into fibrin was investigated by induction of an *in vitro* human plasma clot in the following manner. To a siliconised 5 ml glass vial was added, (a) 800µl of *Tris*(hydroxymethyl)aminomethane buffered saline pH 7.5 containing calcium chloride (50mM Tris, 150mM sodium chloride, 4mM calcium chloride.), (b) about 40µl of physiological salt solution containing 100 units of thrombin per ml, (c) about 400µl of human plasma containing the radiolabelled substrate at a concentration of typically 10kBq/ml. To aid induction of clot a roughened glass rod was added to the reaction vial. Control vials were prepared similarly but with the omission of thrombin and calcium chloride.

After incubation of the test solution at ambient laboratory temperature (ca. 20°C) for 60 minutes the reaction was discontinued with the addition of about 400µl of a cold solution of 33.5mM ethylenediaminetetra-acetic acid disodium salt. Clots were separated from serum by vacuum filtration onto 0.45 µM nitrocellulose filters (pre-soaked in 1.5% BSA/*tris*(hydroxymethyl)aminoethane buffered saline pH 7.5 containing 0.1% Tween 20) and washed with about 2 x 10ml of *tris*(hydroxymethyl)aminomethane buffered saline pH 7.5 containing Tween 20 to a final concentration of 0.1%v/v. The proportion of total radioactivity was calculated by counting in suitable detection apparatus.

The fraction of radioactivity retained on the filter, after subtraction of the non-specific binding determined from the control, is a measure of incorporation into the filtered clots.

Compound	% retained (with thrombin)	% retained (no thrombin)	% specific uptake
^{123}I-Cmpd 1	14.6	2.0	12.6
^{123}I-Cmpd 2	38.6	0.2	38.4
^{99m}Tc-Cmpd 3	39.3	0.3	39.0
^{99m}Tc-Cmpd 4	22.0	0.1	21.0
^{99m}Tc-Cmpd 5	35.5	0.2	35.3
^{99m}Tc-Cmpd 6	68.6	4.3	64.3
^{99m}Tc-Cmpd 7	41.3	0.7	40.6
^{99m}Tc-Cmpd 8	25.9	1.4	24.5
^{99m}Tc-Cmpd 9	44.5	2.7	42.8
^{99m}Tc-Cmpd 10	65.9	0.2	65.7
^{99m}Tc-Cmpd 11	68.1	1.2	66.9
^{99m}Tc-Cmpd 12	34.8	0.3	34.5
^{99m}Tc-Cmpd 13	57.0	0.2	56.8
^{99m}Tc-Cmpd 14	3.3	0.5	2.8
^{99m}Tc-Cmpd 15	55.6	0.4	55.2
^{99m}Tc-Cmpd 16	12.5	0.1	12.4
^{99m}Tc-Cmpd 17	41.3	0.7	40.6
^{99m}Tc-Cmpd 18	9.8	0.1	9.7
^{99m}Tc-Cmpd 19	48.2	0.2	48.0
^{99m}Tc-Cmpd 20	65.0	2.6	62.4
^{99m}Tc-Cmpd 21	60.6	0.5	60.1
^{99m}Tc-Cmpd 22	59.0	0.3	58.7
^{99m}Tc-Cmpd 23	63.8	0.1	63.7
^{99m}Tc-Cmpd 24	56.8	0.4	56.4
^{99m}Tc-Cmpd 25	68.0	4.3	63.7
^{99m}Tc-Cmpd 26	63.3	8.2	55.1
^{99m}Tc-Cmpd 27	Nd	Nd	nd
^{99m}Tc-Cmpd 28	42.1	0.2	41.9
^{99m}Tc-Cmpd 29	21.1	0.1	21.0
^{99m}Tc-Cmpd 30	14.9	0.2	14.7
^{99m}Tc-Cmpd 31	7.7	0.1	7.6

Compound	% retained (with thrombin)	% retained (no thrombin)	% specific uptake*
^{99m}Tc-Cmpd 32	20.2	0.4	19.8
^{99m}Tc-Cmpd 33	63.9	0.1	63.8
^{99m}Tc-Cmpd 34	5.5	0.1	5.4
^{99m}Tc-Cmpd 35	15.9	0.6	15.3
^{99m}Tc-Cmpd 36	8.7	0.1	8.6
^{99m}Tc-Cmpd 37	13.0	0.2	12.8
^{99m}Tc-Cmpd 38	21.0	0.7	20.3
^{99m}Tc-Cmpd 39	23.1	0.1	23.0
^{99m}Tc-Cmpd 40	13.3	0.1	13.2
^{99m}Tc-Cmpd 41	44.5	7.1	37.4
^{99m}Tc-Cmpd 42	14.2	0.2	14.0
^{99m}Tc-Cmpd 43			
^{123}I-Cmpd 44	14.1	0.3	13.8
^{99m}Tc-Cmpd 45	28.7	0.1	28.6
^{99m}Tc-Cmpd 46	6.3	0.2	6.1
^{99m}Tc-Cmpd 47	49.9	0.1	49.8
^{99m}Tc-Cmpd 48	10.6	0.1	10.5
^{99m}Tc-Cmpd 49	33.1	1.6	31.5

* % retained in plasma clot assay (with thrombin) - % retained in plasma clot assay (no thrombin)

Example 16: Normal rat biodistribution

The resolution of a clot image is dependant on the combination of rate of incorporation of the radiopharmaceutical and its blood/tissues clearance rate. For this reason the biodistribution of several compounds has been determined in rats. Male Wistar (100-150g) rats were injected i.v. with 0.1-0.2 ml of radiolabelled tracer solution (8MBq/ml) and dissected at different times post-injection. The %ID in each of the selected tissues was measured. Some animals were kept in metabolism cages to be able to determine the %ID excreted in urine and faeces. The

dissection times used for the agent were 15, 30, 60, 240 min. Data are shown as mean of %ID (n=3).

^{99m}Tc-Compound 3

	15min	30 min	60 min	240 min
Muscle	14.7	10.4	5.1	2.9
Blood	5.3	1.8	1.6	0.6
Kidney	7.6	4.9	4.6	3.4
Urine	14.9	34.3	37.0	42.0
Lung	0.7	0.4	0.3	0.3
Liver	6.2	4.1	4.0	3.0
GI Tract	13.5	15.1	18.0	20.7
Heart	0.2	0.1	0.1	0.04

Example 17: Incorporation into clots induced in a rat model

Rat Inferior vena cava model (IVC)

The rats (Male Wistar,250-350g) were anaesthetised with 15% urethane. After laparotomy, the vena cava was isolated and freed of surrounding fat tissue. A platinum wire (1.5cm x 0.5mm) was inserted into the inferior vena cava and 5 min post surgery 0.4ml of ellagic acid (1.2×10^{-4} M) was injected intravenously through the femoral vein previously canulated, and the clot was allowed to form. The average weight of the clots formed in this model was around 27mg, n=32, (5-50mg range). The compounds were injected 5 min (fresh clot) and 60 min (aged clot) post-induction. After 60 min the animals were sacrificed and the clot removed, weighed and counted. Other tissues e.g. blood, lung, heart, were also dissected and counted. The uptake of tracer into the clot was determined as the relative concentration (cpm/g of clot by dose/g animal)

and clot to background tissue.

Results:

Fresh Clots

Compound	%id/g	Rel. Conc.	Clot/blood	Clot/lung	Clot/ heart	Clot/ liver
^{123}I-Cmpd 1	0.5±0.1	1.6±0.4	1	nd	Nd	nd
^{123}I-Cmpd 2	4.9±0.8	14.5±2.2	7	10	19	21
^{99m}Tc-Cmpd 3	1.4±0.4	5.1±1.2	10	8	17	6
^{99m}Tc-Cmpd 4	2.0±0.6	6.5±2.0	6	8	15	20
^{99m}Tc-Cmpd 5	6.0±2.1	16±5.8	21	23	51	43
^{99m}Tc-Cmpd 7	4.8±1.3	14.5±4.4	15	11	21	6
^{99m}Tc-Cmpd 8	6.7±1.7	21.0±6.8	14	18	41	21
^{99m}Tc-Cmpd 9	8.8±3.6	24.8±9.6	13	12	32	13
^{99m}Tc-Cmpd 10	2.9±0.5	6.5±1.2	4	5	10	5
^{99m}Tc-Cmpd 11	1.1±0.4	3.3±1.1	10	11	24	11
^{99m}Tc-Cmpd 12	7.3±4.1	19.8±11.0	20	17	31	8
^{99m}Tc-Cmpd 13	3.4±0.7	7.9±1.6	7	8	18	18
^{99m}Tc-Cmpd 14	0.5±0.1	1.2±0.2	2	1.5	3	0.7
^{99m}Tc-Cmpd 15	3.6±1.0	8.3±2.2	4	6	12	11
^{99m}Tc-Cmpd 16	0.3±0.1	1.0±0.2	3	3	8	0.8
^{99m}Tc-Cmpd 17	1.2±0.7	3.7±2.3	7	12	18	5
^{99m}Tc-Cmpd 19	2.5±0.3	7.1±1.2	3	7	29	17
^{99m}Tc-Cmpd 20	2.3±0.6	6.5±1.3	12	11	30	18
^{99m}Tc-Cmpd 21	2.3±1.3	7.4±4.0	11	16	28	13
^{99m}Tc-Cmpd 22	3.8±1.6	13.0±5.5	11	13	28	25
^{99m}Tc-Cmpd 23	7.6±1.8	18.2±4.1	6	6	17	21
^{99m}Tc-Cmpd 24	4.0±2.2	11.1±6.7	10	10	21	11
^{99m}Tc-Cmpd 26	14.1±10	41.4±28	16	11	38	16
^{99m}Tc-Cmpd 28	3.5±2.0	9.7±5.0	13	19	31	35
^{99m}Tc-Cmpd 29	4.7±1.9	13.0±4.9	14	20	44	44
^{99m}Tc-Cmpd 31	0.3±0.2	0.9±0.6	0.6	1	2	3

Compound	%id/g	Rel. Conc.	Clot/blood	Clot/lung	Clot/ heart	Clot/ liver
^{99m}Tc-Cmpd 32	1.7±0.7	4.6±1.9	10	11	26	27
^{99m}Tc-Cmpd 33	2.7±0.2	7.8±0.6	11	16	32	28
^{99m}Tc-Cmpd 34	0.5±0.2	1.8±0.5	2	3	6	8
^{99m}Tc-Cmpd 35	1.2±0.2	3.7±0.8	2	2	4	2
^{99m}Tc-Cmpd 36	0.2±0.1	0.7±0.3	1	1	3	4
^{99m}Tc-Cmpd 37	0.4±0.2	0.9±0.4	0.6	0.7	1	1
^{99m}Tc-Cmpd 38	1.9±0.5	5.4±1.5	5	6	15	3
^{99m}Tc-Cmpd 39	2.5±0.3	9.0±1.1	9	14	29	31
^{99m}Tc-Cmpd 41	0.7±0.1	2.2±0.3	3	4	6	8
^{99m}Tc-Cmpd 42	0.3±0.1	0.3±0.2	2	0.3	1	63
^{123}I-Cmpd 44	0.4±0.1	1.1±0.2	0.9	1.5	1	2
^{99m}Tc-Cmpd 45	2.0±0.5	5.5±1.3	6	5	10	3
^{99m}Tc-Cmpd 46	0.8±0.4	2.0±0.7	5	3	6	1
^{99m}Tc-Cmpd 47	0.8±0.7	2.2±2.0	0.8	1	3	1
^{99m}Tc-Cmpd 48	0.3±0.2	1.0±0.5	0.6	0.9	2	3
^{99m}Tc-Cmpd 49	2.6±1.1	8.5±3.7	4	6	16	4

Rel. Conc.(RC) = $\frac{\text{\%id/g of clot}}{\text{\%id/g in rest of body}}$

Aged Clots

Compound	%id/g	Rel. Conc.	Clot/blood	Clot/lung	Clot/ heart	Clot/ liver
^{123}I-Cmpd 2	5.5±1.7	14.5±3.9	24	12	23	20
^{99m}Tc-Cmpd 3	2.1±0.8	6.2±2.2	8	11	23	5
^{99m}Tc-Cmpd 4	1.2±1.1	4.1±4.0	8	10	19	19
^{99m}Tc-Cmpd 5	3.6±1.7	11±5.1	13	31	33	24
^{99m}Tc-Cmpd 7	2.1±0.3	6.5±1.3	9	7	15	3
^{99m}Tc-Cmpd 8	5.3±1.3	16.4±4.3	10	9	24	16
^{99m}Tc-Cmpd 9	3.7±0.4	11.4±1.5	8	7	21	6
^{99m}Tc-Cmpd 10	2.9±1.4	6.3±2.7	6	6	16	5
^{99m}Tc-Cmpd 11	0.4±0.3	1.2±0.8	5	6	13	4
^{99m}Tc-Cmpd 12	4.1±1.2	11.2±3.4	19	15	75	7

Compound	%id/g	Rel. Conc.	Clot/blood	Clot/lung	Clot/ heart	Clot/ liver
^{99m}Tc-Cmpd 13	4.5±1.5	10.4±3.0	6	9	29	19
^{99m}Tc-Cmpd 14	0.6±0.4	1.4±0.8	1.4	1.3	2	0.7
^{99m}Tc-Cmpd 15	3.4±0.6	7.6±1.6	3	0.6	1	nd
^{99m}Tc-Cmpd 17	0.8±0.1	2.7±0.6	3	5	7	3
^{99m}Tc-Cmpd 19	3.2±2.3	9.1±7.1	14	5	13	6
^{99m}Tc-Cmpd 20	1.7±0.5	4.9±1.4	10	14	36	23
^{99m}Tc-Cmpd 21	2.7±2.3	8.5±6.8	31	13	32	31
^{99m}Tc-Cmpd 22	6.9±2.5	22.7±7.4	11	1	3	4
^{99m}Tc-Cmpd 23	7.5±2.5	17.6±4.6	13	1	1	1
^{99m}Tc-Cmpd 24	4.1±2.5	11.9±8.0	15	20	40	37
^{99m}Tc-Cmpd 26	4.0±0.9	12.9±3.5	7	12	31	54
^{99m}Tc-Cmpd 28	4.5±1.1	13.1±3.4	17	2	3	1
^{99m}Tc-Cmpd 29	3.8±0.4	10.0±1.7	11	30	67	29
^{99m}Tc-Cmpd 31	0.2±0.1	0.6±0.2	0.6	4	9	2
^{99m}Tc-Cmpd 32	0.7±0.1	2.0±0.4	3	15	43	21
^{99m}Tc-Cmpd 33	2.2±0.6	6.7±1.6	16	9	24	16
^{99m}Tc-Cmpd 34	0.4±0.1	1.4±0.4	3	-	-	6
^{99m}Tc-Cmpd 35	0.6±0.1	1.5±0.4	1	0.5	1	2
^{99m}Tc-Cmpd 36	0.1±0.02	0.2±0.1	0.6	10	18	4
^{99m}Tc-Cmpd 37	0.2±0.03	0.5±0.1	0.4	1	2	9
^{99m}Tc-Cmpd 38	0.8±0.3	2.4±0.7	3	0.5	1	2
^{99m}Tc-Cmpd 39	2.4±0.3	8.2±1.1	15	12	43	11
^{99m}Tc-Cmpd 41	0.4±0.1	1.1±0.3	1	6	13	4
^{99m}Tc-Cmpd 42	0.4±0.1	1.3±0.3	5	7	21	6
^{123}I-Cmpd 44	0.5±0.03	1.2±0.1	0.9	2	1	1
^{99m}Tc-Cmpd 45	2.0±0.8	5.7±2.3	8	5	11	3
^{99m}Tc-Cmpd 46	0.5±0.2	1.3±0.6	4	3	5	1
^{99m}Tc-Cmpd 47	0.5±0.3	1.5±0.8	1	24	46	44
^{99m}Tc-Cmpd 48	0.2±0.04	0.8±0.2	1	109	32	26
^{99m}Tc-Cmpd 49	1.2±0.5	3.9±1.6	4	9	29	19

Example 18: Imaging of clots induced in a rat model

Clots were induced in male Wistar rats (250-350g) as described in Example 17, with the exception that for these experiments the platinum wire was located in the jugular vein. The compounds were injected 60 min post injection and planar images acquired between 15-180 min p.i. A Park medical Isocam I gamma camera was used for these experiments, 300K or 150K counts were of the thorax were collected using a LEUHR or a LEPH collimator. Clots were visualised from 15 min p.i. Reaching the highest clot to background ratio at 180 min p.i. due to rapid clearance of the compound.

Abbreviations

Ac	Acetyl
Boc	tert-butyloxycarbonyl
Cmpd	compound
DMF	dimethylformamide
ES	electrospray
FAB	fast atom bombardment
Fmoc	fluorenylmethoxycarbonyl
HPLC	high performance liquid chromatography
MALDI-TOF	matrix assisted laser desorption ionisation – time of flight
Nal	napthylalanine
RCP	radiochemical purity
RP-HPLC	reverse phase high performance liquid chromatography
TFA	trifluoroacetic acid
TLC	thin layer chromatography

CLAIMS

1. A compound of formula:

$Y\text{-}(CR_2)_n\text{-}X\text{-}NHJ$

where:

X is C=O or CR_2;

n is an integer of value 1 to 6;

Y is $L(A)_m$- or R^1R^2CR-where L is a metal complexing agent,

A is $-CR_2$-, -CR=CR-, -C≡C-, -NRCO-, -CONR-, SO_2NR-, $-NRSO_2$-, $-CR_2OCR_2$-, $-CR_2SCR_2$-, $-CR_2NRCR_2$-, a C_{4-8} cycloheteroalkylene group, a C_{4-8} cycloalkylene group, a C_{5-12} arylene group, a C_{3-12} heteroarylene group or a polyalkyleneglycol, polylactic acid or polyglycolic acid moiety;

m is an integer of value 0 to 10;

where one of R^1 and R^2 is $-NH(B)_pZ^1$ and the other is $-CO(B)_qZ^2$ where

p and q are integers of value 0 to 45, and

each B is independently chosen from Q or an amino acid residue,

where Q is a cyclic peptide;

Z^1 and Z^2 are protecting groups which are biocompatible groups which inhibit or surpass *in vivo* metabolism of the peptide.

J and each R group are independently chosen from H, C_{1-4} alkyl, C_{1-4} alkenyl, C_{1-4} alkynyl, C_{1-4} alkoxyalkyl or C_{1-4} hydroxyalkyl;

with the provisos that:

(i) the total number of amino acid residues in the R^1 and R^2 groups does not exceed 45;

(ii) when X is CR_2, then Y is $-CRR^1R^2$ and Z^2 is a metal complexing agent;

(iii) when Y is $-CRR^1R^2$ then at least one of R^1 and R^2 bears at least one detectable moiety.

2. The compound of claim 1 where R^1 or R^2 includes one or more peptide fragments of α_2-antiplasmin, fibronectin, beta-casein, tetanus, amyloid, trappin and polyglutamine residues, said peptide fragment containing at least three amino acid residues.

3. The compound of claim 2 where the peptide fragment is from α_2-antiplasmin.

4. The compound of claim 3 where the amino acid in the 2-position from the peptide N-terminus is glutamine.

5. The compound of claims 1 to 4 where J is H.

6. The compound of claim 5 of formula:

$Y\text{-}(CR_2)_x\text{-}(CH_2)_2CONH_2$ or $Y\text{-}(CR_2)_y\text{-}(CH_2)_4NH_2$

where x is an integer of value 0 to 4, and y is an integer of value 0 to 3.

7. The compound of any one of claims 1 to 6 where Y is $-CRR^1R^2$.

8. The compound of any one of claims 1 to 7 where at least one of Z^1 and Z^2 is a metal complexing agent.

9. The compound of claim 8 where Z^2 is a metal complexing agent and Z^1 is not a metal complexing agent.

10. A metal complex of the compounds of claim 8 or claim 9.

11. The metal complex of claim 10 where the metal is a radiometal.

12. The radiometal complex of claim 11 where the radiometal is ^{99m}Tc.

13. A preparation for human administration comprising the compound of any one of claims 1 to 12.

14. A kit comprising the compound of any one of claims 1 to 9 useful in the preparation of the metal complexes of any one of claims 10 to 12.

15. Use for the diagnosis of sites of thrombosis or embolism of a compound of formula:

$$Y\text{-}(CR_2)_n\text{-}X\text{-}NHJ$$

where:

X is C=O or CR_2;

n is an integer of value 1 to 6;

Y is $L(A)_m$- or R^1R^2CR- where L is a metal complexing agent,

A is $-CR_2$- , -CR=CR- , -C≡C- , -NRCO- , -CONR- , $-SO_2NR$- , $-NRSO_2$- , $-CR_2OCR_2$- , $-CR_2SCR_2$- , $-CR_2NRCR_2$- ; a C_{4-8} cycloheteroalkylene group, a C_{4-8} cycloalkylene group, a C_{5-12} arylene group, a C_{3-12} heteroarylene group or a polyalkyleneglycol, polylactic acid or polyglycolic acid moiety;

m is an integer of value 0 to 10;

where one of R^1 and R^2 is $-NH(B)_pZ^1$ and the other is $-CO(B)_qZ^2$ where

p and q are integers of value 0 to 45, and

each B is independently chosen from Q or an amino acid residue,

where Q is a cyclic peptide;

Z^1 and Z^2 are protecting groups;

J and each R group are independently chosen from H, C_{1-4} alkyl, C_{1-4} alkenyl, C_{1-4} alkynyl, C_{1-4} alkoxyalkyl or C_{1-4} hydroxyalkyl;

with the provisos that:

(i) the total number of amino acid residues in the R^1 and R^2 groups does not exceed 45;

(ii) when X is CR_2, then Y is $-CRR^1R^2$;

(iii) when Y is $-CRR^1R^2$ then at least one of R^1 and R^2 bears at least one detectable moiety.

16. Use for the diagnosis of sites of thrombosis or embolism of a radiometal complex of the compound defined in claim 13, wherein at least one of Z^1 and Z^2 is a metal complexing agent.

17. A peptide fragment of α_2-antiplasmin, fibronectin, beta-casein, tetanus, amyloid, trappin or polyglutamine, said peptide fragment containing 3 – 45 amino acid residues and carrying a terminal metal complexing agent.

18. The peptide fragment of claim 17, wherein the metal complexing agent is at the carboxy terminus.

19. A metal complex of the peptide fragment of claim 17 or claim 18.

Figure 1: Imaging with ^{99m}Tc-Compound 5.

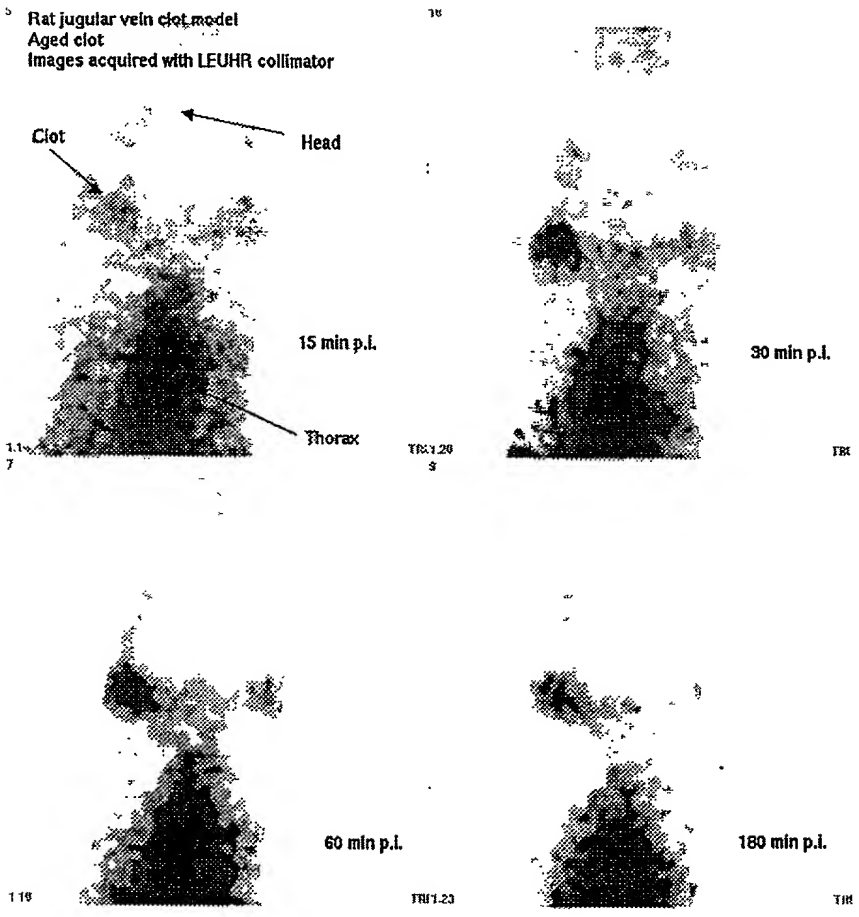
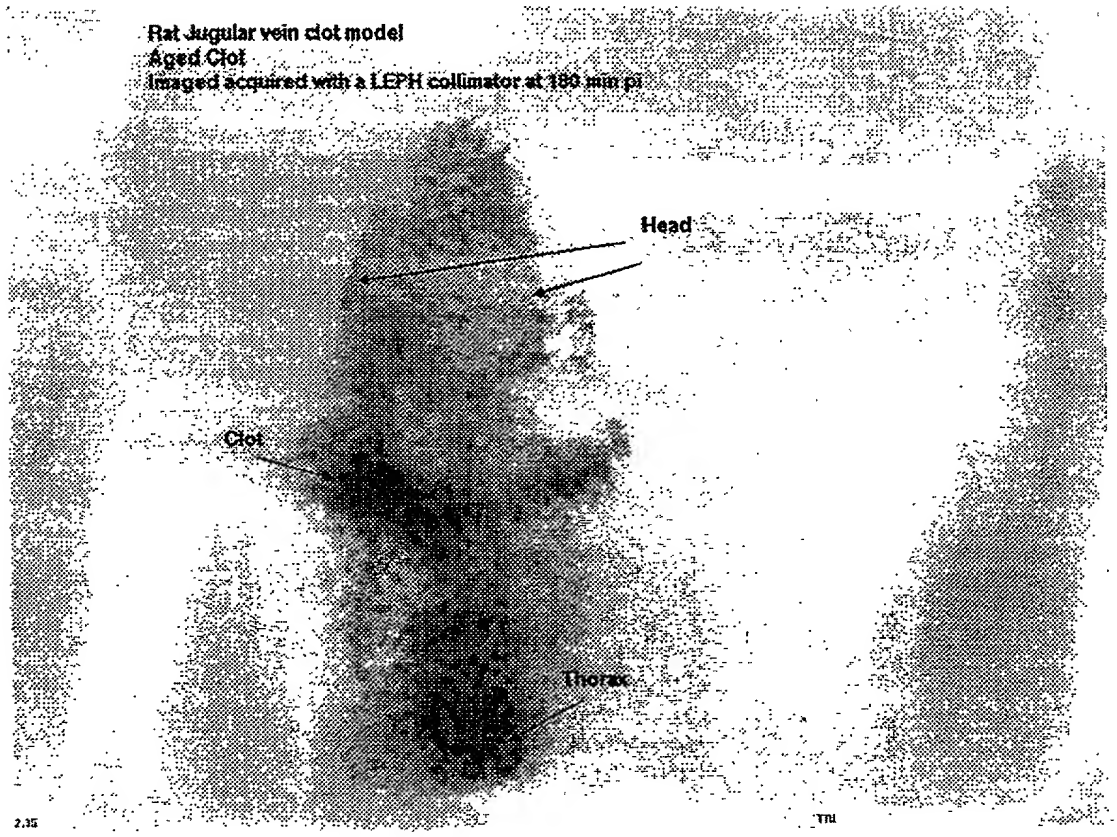


Figure 2: Imaging with ^{99m}Tc-Compound 5.



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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	PA-9816
First Named Inventor	Storey
COMPLETE IF KNOWN	
Application Number	09 / 674,616
Filing Date	1-Nov-2000
Group Art Unit	To be assigned
Examiner Name	To be assigned

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Labelled Glutamine and Lysine Analogues

(Title of the Invention)

the specification of which
☐ is attached hereto
OR
☒ was filed on (MM/DD/YYYY) 11/01/2000 as United States Application Number or PCT International Application Number 09/674,616 and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
98303872.0	EP	05/15/1998	☐	☐	☒
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below

Application Number(s)	Filing Date (MM/DD/YYYY)

☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

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DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
PCT/GB99/01550	05/14/1999	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

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Address					
City		State		ZIP	
Country		Telephone		Fax	

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Anthony Eamon	Storey

Inventor's Signature	X Anthony Storey GBN					Date	X 21/11/00
Residence: City		State		Country	GB	Citizenship	GB
Post Office Address	~~Lynwood, The Common, Winchmore Hill, Amersham~~ THE CHERRIES, MIDDLE STREET, STAINBY						
Post Office Address	~~Buckinghamshire HP7 0PN~~ GRANTHAM, LINCS. NG33 5QZ. Great Britain						
City		State		ZIP		Country	

☒ Additional inventors are being named on the 2 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

Please type a plus sign (+) inside this box → [+]

PTO/SB/02A (3-97)
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ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 1 of 2

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])			Family Name or Surname				
Marivi			Mendizabal				
Inventor's Signature X					**Date** X	21 NOV 2000	
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Post Office Address	56 Springfield Avenue, Merton Park, London						
Post Office Address	Greater London SW20 9JX Great Britain						
City		**State**		**ZIP**		**Country**	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])			Family Name or Surname				
Susan			Champion				
Inventor's Signature X					**Date** X	22 Nov 2000	
Residence: City		**State**		**Country** GB		**Citizenship**	GB
Post Office Address	~~20 Lindo Close, Chesham~~ 68 GROVE GARDENS, TRING HERTS. HP23 5PY						
Post Office Address	~~Buckinghamshire HP5 2JP~~ Great Britain						
City		**State**		**ZIP**		**Country**	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])			Family Name or Surname				
Alex			Gibson				
Inventor's Signature X					**Date** X	22 Nov 2000	
Residence: City		**State**		**Country** GB		**Citizenship**	GB
Post Office Address	~~24 Farm Close, Little Chalfont~~ 67 Drovers Court, Troy Close Oxford, Oxfordshire						
Post Office Address	~~Buckinghamshire HP6 6RH~~ Great Britain OX3 7SQ						
City		**State**		**ZIP**		**Country**	

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ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 2 of 2

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])	Family Name or Surname						
Benedicte	Guilbert						
Inventor's Signature X	B Guilbert					Date X	22/11/00
Residence: City		State		Country GB		Citizenship	FR
Post Office Address	4 MULBERRY CLOSE, WATFORD ~~14 Chesswood Court, Bury Lane, Rickmansworth~~						
Post Office Address	Hertfordshire ~~WD3 1DF~~ WD1 3UZ Great Britain						
City		State		ZIP		Country	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])	Family Name or Surname						
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Inventor's Signature X	Ian Wilson					Date X	22/11/00
Residence: City		State		Country GB		Citizenship	GB
Post Office Address	18 Sturrock Way, Hitchin						
Post Office Address	Hertfordshire SG4 0EP Great Britain						
City		State		ZIP		Country	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])	Family Name or Surname						
Peter	Knox						
Inventor's Signature						Date	
Residence: City		State		Country GB		Citizenship	GB
Post Office Address	"Choppings", 34 Kings Road, Chalfont St. Giles						
Post Office Address	Buckinghamshire HP8 4HS Great Britain						
City		State		ZIP		Country	

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ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 2 of 2

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])		Family Name or Surname					
Benedicte		Guilbert					
Inventor's Signature						**Date**	
Residence: City		**State**		**Country**	GB	**Citizenship**	FR
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Post Office Address	Hertfordshire WD3 1DF Great Britain						
City		**State**		**ZIP**		**Country**	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])		Family Name or Surname					
Ian Andrew		Wilson					
Inventor's Signature						**Date**	
Residence: City		**State**		**Country**	GB	**Citizenship**	GB
Post Office Address	18 Sturrock Way, Hitchin						
Post Office Address	Hertfordshire SG4 0EP Great Britain						
City		**State**		**ZIP**		**Country**	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])		Family Name or Surname					
Peter		Knox					
Inventor's Signature X	Peter K					**Date** X	27.11.00
Residence: City		**State**		**Country**	GB	**Citizenship**	GB
Post Office Address	"Choppings", 34 Kings Road, Chalfont St. Giles						
Post Office Address	Buckinghamshire HP8 4HS Great Britain						
City		**State**		**ZIP**		**Country**	

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